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Original article

Design, synthesis and antiepileptic properties of novel 1-(substituted benzylidene)-3-(1-(morpholino/piperidino methyl)-2,3-dioxoindolin-5-yl) urea derivatives

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ABSTRACT

Twenty new 1-(substituted benzylidene)-3-(1-(morpholino/piperidino methyl)-2,3-dioxoindolin-5-yl) urea derivatives were designed and synthesized. Antiepileptic screening was performed using MES and *sc*PTZ seizures tests. The neurotoxicity was determined by rotorod test. In the preliminary screening, compounds **5c**, **5g**, **5j** and **5n** were found active in MES model, while **5o** showed significant antiepileptic activity in *sc*PTZ model. Further all these five compounds were administered orally to rats, **5c**, **5g** and **5n** showed better activity than Phenytoin in oral route. Among these compounds **5c** revealed protection in MES at a dose of 30 mg/kg and 100 mg/kg 0.5 h and 4 h after *i.p.* administration respectively. This molecule provided also protection in the *sc*PTZ at a dose of 300 mg/kg in both time intervals.

1. Introduction

Epilepsy is one of the more common brain disorder characterized by recurrent spontaneous seizures of cerebral origin, presenting with episodes of sensory, motor or autonomic phenomenon with or without loss of consciousness [1]. Epilepsy affects approximately 1% of the world's population according to epidemiological studies, and often, therapeutic regimens for epileptic patients will involve a change of first line and/or add-on antiepileptic drugs [2].The currently used antiepileptic drugs can be broadly classified into four categories on the basis of the main molecular mechanisms of action, as follow: (a) enhancement of GABA mediated inhibition or other effect on the GABA system, (b) modulation of voltage-dependent Na^+ and/or Ca^{2+} channels, (c) modulation of synaptic release and (d) inhibition of synaptic excitation mediated by ionotropic glutamate receptors [3]. The efficacy of many of the marketed antiepileptic drugs is greatly compromised by severe side effects such as ataxia, drowsiness, gingival hyperplasia, gastrointestinal disturbances, and megaloblastic anaemia, Moreover about 30% of patients have uncontrolled seizures [4–6]. The insufficient information on the cellular mechanism of epilepsy in human and the complex mechanism of action of most of the antiepileptic drugs makes it difficult to use rational methodologies in the field of drug discovery. Therefore an another design of new antiepileptics is based on the existence of different pharmacophores that were established through the analysis of structural characteristics of clinically effective drugs as well as other antiepileptic compounds [7–9]. In the literatures [10,11], it is well documented that one of the important core fragments is defined by presence of

i) Hydrogen donor/Acceptor unit (HAD),
ii) One electron donor atom, (D) and
iii) A hydrophobic domain (A) (aryl ring substituted/ Unsubstituted)

This common template was found in the structures of first generation, however well-established antiepileptics such as carbamazepine or phenytoin, among the newest drugs e.g. Felbamate or in the second generation antiepileptic drugs and the drugs in clinical trial (Fig. 1). Much efforts devoted in the recent years based on the pharmacophore model for the development of novel therapeutics resulted in the availability of several newer drugs (such as tiagabine, lamotrigine, pregabalin, stiripentol, zonisamide, topiramate, levetiracetam) as promising antiepileptics [12]. Therefore, continued search for novel antiepileptic drugs with less toxicity and more selectivity to be an area of investigation in the field of medicinal chemistry.

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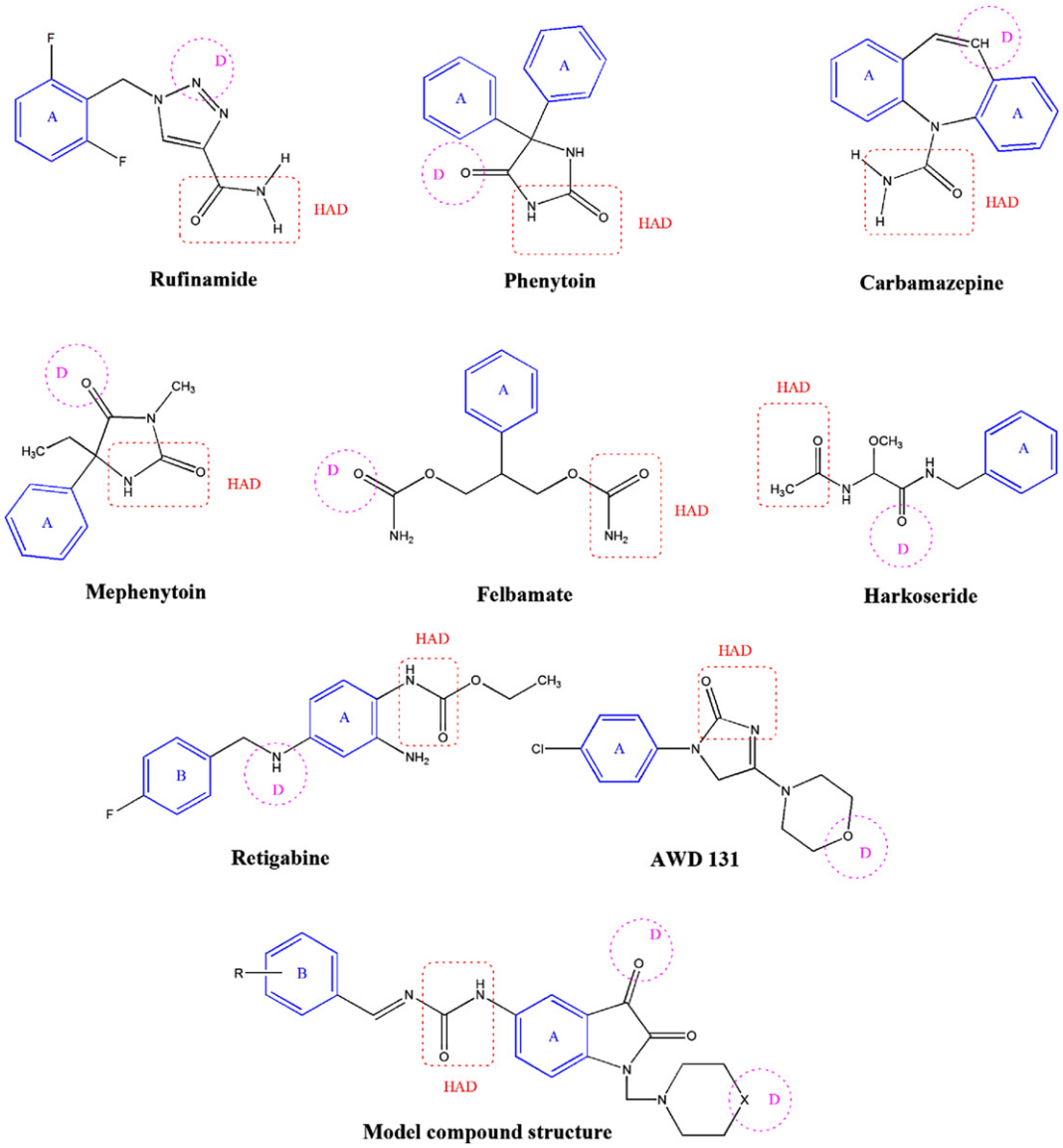


Fig. 1. Pharmacophoric pattern of well known antiepileptics and model compound with its vital structural features: (A) hydrophobic aryl ring system, (HAD) hydrogen bond acceptor/donor domain, (D) electron donor moiety and (B) distal aryl ring.

Many studies revealed that Isatin is a privileged lead molecule for scheming potential bioactive agents, and their derivatives constitute an important class of heterocyclic compounds and are shown to possess a broad spectrum of bioactivity as many, such as anticonvulsant [13], anti-HIV [14], anti-viral [15], anti-tumor [16], anti-angiogenic [17], anti-fungal [18], anti-malarial [19], anti-oxidant [20], anti-glycation [21], and potent inhibitors against caspase-3 [22]. These exciting properties encouraged many efforts toward the synthesis and pharmacological screening of many isatin derivatives.

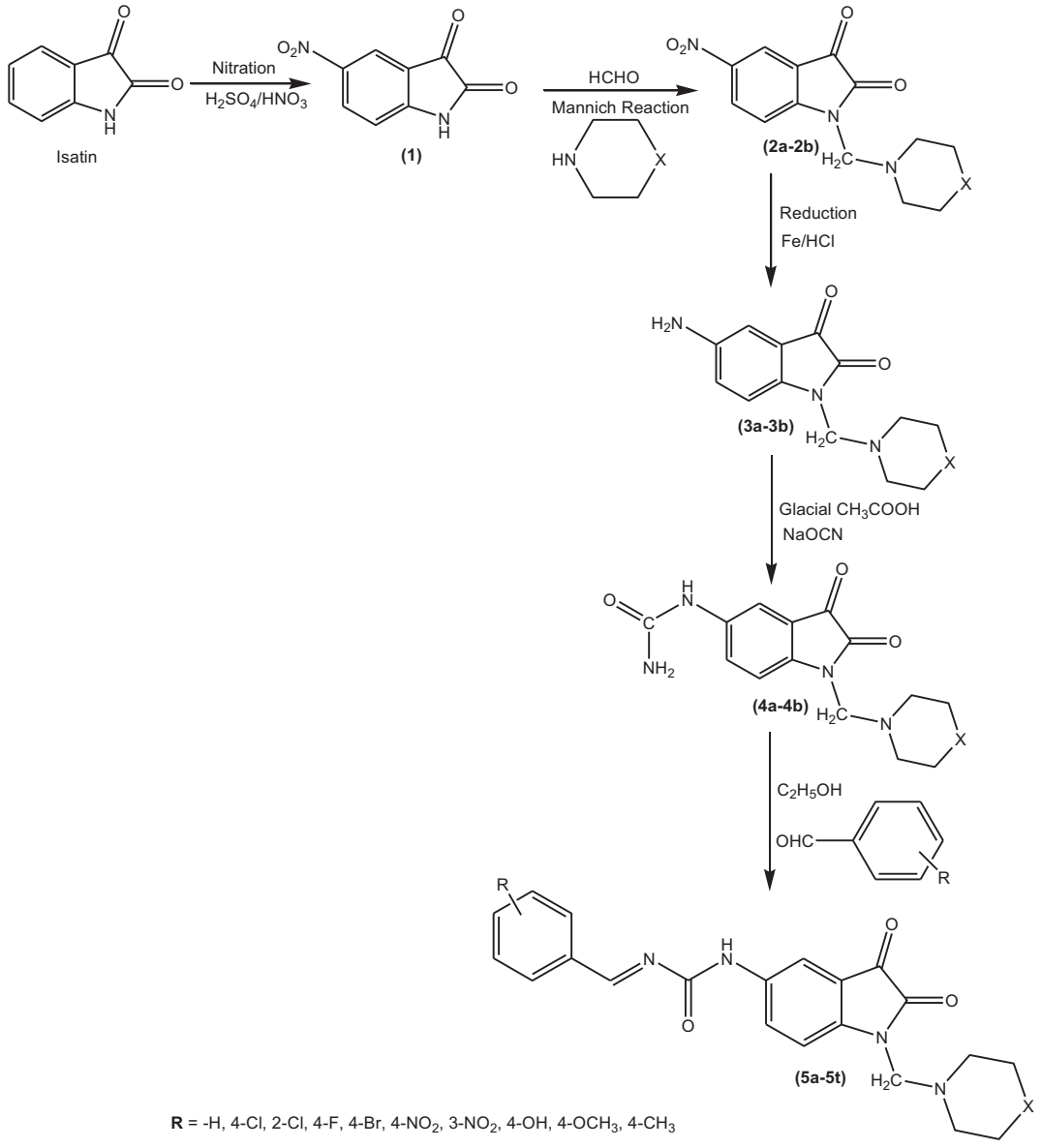
Based on the above literature review and considering the wide applications of isatin molecule in medicinal chemistry an attempt has been made to synthesize different substituted benzylidene urea derivatives containing isatin moiety as antiepileptic agents.

2. Chemistry

The synthetic pathway giving access to the titled compounds **(5a–5t)** was illustrated in Scheme 1. The synthesis of 5-nitro isatin (**1**) involved a simple nitration of starting material by nitric acid and sulphuric acid. In the subsequent step, 5-nitro-1-substituted indolin-2,3-dione (**2**) was synthesized through Mannich reaction by treating compound **1** with formaldehyde and secondary amines like morpholine and piperidine. Compounds (**2a**,**b**) get reduced by heating with a mixture of hydrochloric acid and iron powder, to offer 5-amino-1-substituted indolin-2,3-dione (**3a**,**b**). On stirring with sodium cyanate and glacial acetic acid, compounds (**3a**,**b**) get converted to its respective urea derivatives. In the last step, the compounds (**5a–5t**) were synthesized by a Schiff base reaction, in which a different aromatic aldehydes (carbonyl compound) and amino derivative (Isatin Mannich base analog) undergoes nucleophilic addition, forming a hemiaminal. This reaction is followed by a dehydration to generate a title compounds (**5a–5t**) by forming a stable imine. TLC was performed throughout the reactions to optimize the reactions for purity and completion. The physicochemical parameters of all the synthesized compounds are summarized in Table 1.

3. Pharmacology

All the synthesized compounds were evaluated for their anti-epileptic effects using male albino mice (Swiss, 18–25 g) and rat



Scheme 1. Synthetic protocols of target compounds **5a**–**5t**.

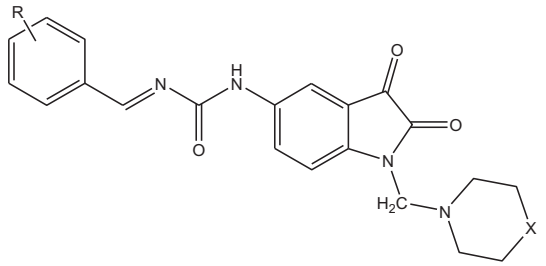
(Wistar 100–150 g). The primary qualitative evaluations were performed in mice involved two epilepsy tests (MES: Maximal Electroshock Seizure test and *Sc*PTZ: Subcutaneous pentylenetetrazole). Acute neurological toxicity induced by the compounds in mice was assessed through standardized rotorod test. In the initial screening, candidate compounds were screened for their antiepilepsy potential through MES and *sc*PTZ models in mice at a dose level of 30, 100 and 300 mg/kg by intraperitoneal (*i.p*) route and the groups of mice are tested at different time points (i.e., 0.5 and 4 h) post administration of the test candidate. It is generally acknowledged that the MES model, which uses an electrical stimulus, induces generalized tonic-clonic seizures. Through electrical induction, it is used to help recognize those compounds which prevent seizure spread. The *sc*PTZ is a model where the myoclonic seizures induced by chemical induction. It helps in identifying those compounds that might act by increasing seizure threshold.

4. Results and discussion

4.1. Chemistry

The structures of the synthesized compounds were confirmed by elemental analyses and spectral (IR, ^{1}H NMR, and Mass) data. The presence of nitro group in compound **1** is characterized by the presence of two strong bands in its IR spectrum at 1570 cm^{-1} and 1348 cm^{-1} which arises from the asymmetrical and symmetrical stretching modes. The formation of compound **2a** and **2b** were confirmed by the appearance of singlet between 4.28 and 4.40 for two protons in its ^{1}H NMR spectra which might be assigned to CH_2 group connecting the isatin and the secondary amines through Mannich reaction. The conversion of amine (**3a** and **3b**) from nitro (**2a** and **2b**) can be recognized by strong absorption peak at 3386 and 3398 cm^{-1} in IR due to N–H Stretching. The IR spectrum of title compound **5c** over the 3058 cm^{-1} showed multiple weak

Table 1
Physicochemical characterization of compounds **5a**–**5t**.



Compound	R	X	Mol. formula	% yield	Mp (°C)	R_f[a]	Log p[b]
5a	H	O	$C_{21}H_{20}N_4O_4$	68	240–242	0.81	1.94
5b	H	CH_2	$C_{22}H_{22}N_4O_3$	73	256–258	0.90	3.07
5c	4-Cl	O	$C_{21}H_{19}ClN_4O_4$	55	215–217	0.68	2.50
5d	4-Cl	CH_2	$C_{22}H_{21}ClN_4O_3$	61	268–270	0.79	3.63
5e	2-Cl	O	$C_{21}H_{19}ClN_4O_4$	52	235–237	0.70	2.50
5f	2-Cl	CH_2	$C_{22}H_{21}ClN_4O_3$	56	255–257	0.73	3.63
5g	4-F	O	$C_{21}H_{19}FN_4O_4$	66	263–265	0.76	2.10
5h	4-F	CH_2	$C_{22}H_{21}FN_4O_3$	72	244–246	0.65	3.23
5i	4-NO_2	O	$C_{21}H_{19}N_5O_6$	58	168–170	0.60	–
5j	4-NO_2	CH_2	$C_{22}H_{21}N_5O_5$	69	269–271	0.87	0.56
5k	3-NO_2	O	$C_{21}H_{19}N_5O_6$	60	226–228	0.84	–
5l	3-NO_2	CH_2	$C_{22}H_{21}N_5O_5$	62	282–284	0.77	0.56
5m	4-Br	O	$C_{21}H_{19}BrN_4O_4$	74	289–291	0.71	2.77
5n	4-Br	CH_2	$C_{22}H_{21}BrN_4O_3$	70	239–241	0.89	3.90
5o	4-OH	O	$C_{21}H_{20}N_4O_5$	72	185–187	0.91	1.55
5p	4-OH	CH_2	$C_{22}H_{22}N_4O_4$	58	207–208	0.62	2.68
5q	4-OCH_3	O	$C_{22}H_{22}N_4O_5$	77	228–230	0.60	1.81
5r	4-OCH_3	CH_2	$C_{23}H_{24}N_4O_4$	55	196–197	0.78	2.95
5s	4-CH_3	O	$C_{22}H_{22}N_4O_4$	74	183–185	0.69	2.43
5t	4-CH_3	CH_2	$C_{23}H_{24}N_4O_3$	58	175–177	0.75	3.56

[a] Solvent system used was toluene/ethyl acetate/formic acid (5:4:1).
[b] Log P was calculated using ACD lab version 8.0.

absorption peaks corresponding to Ar–H stretching vibration. The strong absorption at 1726 cm^{-1} is due to the C=O stretching vibration and the moderate intensity absorption at 1588 cm^{-1} corresponds to a CH=N stretching vibration. The strong stretching vibration at 850 cm^{-1} arises due to presence of C–Cl bond. It's ^1H NMR spectrum showed a singlet at δ 8.78 ppm due to the proton attached to the imine carbon. A group of signals appeared between δ 6.88 and 8.10 ppm corresponds to Ar–H protons. The presence of CH=N stretching vibration at 1588 cm^{-1} in IR spectrum and a singlet for a proton attached to the imine carbon at δ 8.78 ppm in ^1H NMR confirms the formation of **5c**. Further mass spectrum confirmed their purity and molecular weight.

4.2. Antiepileptic activity

For the identification of antiepileptic activity in mice, test compounds were administered *i.p.* and challenged by MES and *sc*PTZ. Compounds found to be active in these seizure challenges are generally regarded to be significantly useful candidates in treatment of partial, generalized and even absence seizures. The data regarding the antiepileptic screening of all the compounds are reported in Table 2.

In the electroshock investigation, four compounds **5c**, **5g**, **5j** and **5n** were found to be significantly active as they showed protection at the lowest dose of 30 mg/kg after 0.5 h. These compounds continued to show the activity after 4.0 h but at higher doses (100 mg/kg) except **5n**, which needs 300 mg/kg indicating the rapid onset as well as long duration of action of these compounds. The promising nature of the compounds may be attributed to the substitutions at the hydrophobic domain. These compounds had

Table 2
Antiepileptic activity and neurotoxicity of compounds **5a**–**5t** administered intraperitoneally to mice.

Compound	MES[a] screening		*sc*PTZ[b] screening		NT[c] screening	
	0.5 h[d]	4.0 h[d]	0.5 h[d]	4.0 h[d]	0.5 h[d]	4.0 h[d]
5a	–	300	–	–	ND	ND
5b	–	–	300	–	ND	ND
5c	30	100	300	300	–	–
5d	100	300	300	–	–	300
5e	100	300	300	–	300	–
5f	100	300	300	–	100	300
5g	30	100	100	300	300	–
5h	100	300	100	–	300	300
5i	100	–	–	300	–	–
5j	30	100	300	300	300	300
5k	–	300	–	–	ND	ND
5l	–	–	–	300	ND	ND
5m	100	300	300	–	–	–
5n	30	300	–	300	100	300
5o	300	–	>30[e]	300	–	300
5p	300	–	–	300	–	–
5q	–	–	–	300	300	300
5r	–	300	300	–	ND	ND
5s	300	–	–	300	–	–
5t	–	–	300	–	ND	ND
Phenytoin[f]	30	30	–	–	100	100
Ethosuximide[g]	–	–	100	300	–	–

The sign — (mdash) represents an absence of activity at maximum dose administered (300 mg/kg).
ND - Not determined.
[a] Maximal electroshock test (administered intraperitoneally to mice at doses ranging from 30 to 300 mg/kg).
[b] Subcutaneous pentylenetetrazole test (administered intraperitoneally to mice at doses ranging from 30 to 300 mg/kg).
[c] Neurotoxicity (administered intraperitoneally to mice at doses ranging from 30 to 300 mg/kg).
[d] Time of test after drug administration.
[e] scPTZ activity >30 means that the compound showed activity between 30 and 100 mg/kg.
[f] Reference drug, data for phenytoin ref [23].
[g] Reference drug, data for ethosuximide ref [24].

electron withdrawing groups at the para position of the hydrophobic aryl ring. In general it was observed that the para substituted derivatives were more active than the other derivatives. This may be because of the fact that the para substituted derivatives are better fitted into the receptor site. Compounds that showed protection at 100 mg/kg after 0.5 h were **5d**, **5e**, **5f**, **5h**, **5i,** and **5m** indicating the ability of these compounds to protect from seizures at relatively lower dose. These compounds except **5i** were also active after 4.0 h at 300 mg/kg dose. The higher dose required for longer duration of activity may be because of the high lipophilicity of the compounds as it results into bidirectional movements of the compounds through the blood–brain barrier.

In the chemoshock investigation, most of the compounds showed moderate to good antiepileptic activity. Compounds that revealed protection in the *sc*PTZ test, indicative the ability of substance to increasing seizure threshold, at a dose of ≤100 mg/kg after 0.5 h included **5g**, **5h**, and **5o**. It was comparable to results obtained for ethosuximide which is recognized as reference antiepileptic for this screen. Among all compounds, **5o** was found to be remarkably active between 30 and 100 mg/kg dose after 0.5 h, as it continued to show activity at 300 mg/kg after 4.0 h. Other compounds except **5a** and **5k** that showed considerable antiepileptic activity were **5b**–**5f**, **5i**, **5j**, **5l**–**n** and **5p**–**t** at 300 mg/kg either after 0.5 h or 4.0 h. It was observed that in this method, the most active compound have substitution at the para position of the distal aryl ring by electron releasing group resulted in increased antiepileptic activity.

Majority of the compounds that is, **5c**–**j** and **5m**–**5o** were showed activity in either MES or *sc*PTZ model at 30 or 100 mg/kg dose after 0.5 h. The reports indicating that 80% of the compounds that is **5a**, **5c**–**5k**, **5m**–**5p**, **5r** and **5s** were shown activity in MES screening, whereas 90% of the compounds that is, except **5a** and **5k** were active in *sc*PTZ test at any one of the tested dose. These results revealed that maximum of compounds possessed some *sc*PTZ selectivity.

4.3. Neurotoxicity screen

The results obtained showed that most of the candidate compounds exhibited neurotoxicity at doses higher than widely prescribed drugs Phenytoin or Carbamazepine. But while evaluating an antiepileptic compounds, separation between antiepileptic and neurotoxic dose is desirable. All the compounds evaluated for its neurotoxicity study except **5a**, **5b**, **5k**, **5l**, **5r** and **5t**, due to its poor response in antiepileptic activity. In neurotoxic study **5f** and **5n** were found to be neurotoxic at 100 mg/kg. Compounds **5d**, **5e**, **5g**, **5h**, **5j**, **5o** and **5q** were showed neurotoxicity at 300 mg/kg, while all other compounds **5c**, **5i**, **5m**, **5p** and **5s** were not found to be neurotoxic at maximum administered dose.

4.4. Antiepileptic activity of selected compounds on rats by oral administration

A valuable property of a candidate antiepilepsy is its ability to inhibit epilepsy when given by the oral route. This screen discloses the time of onset, the approximate time of peak effect (TPE) and the duration of antiepileptic activity or neurotoxicity. From the initial screen we identified five compounds that were further evaluated for oral availability using the MES acute seizure model and neurotoxicity in rats at a dose of 30 mg/kg. The compound includes **5c**, **5g**, **5j**, **5n** and **5o**. The results obtained are presented in Table 3.

As can be seen from these data, the most active compounds are **5c, 5g** and **5n** which protected 100% (4/4) of rats at time points 2 h and 4 h, 50% (2/4) at 0.5 h and 1 h, 25% (1/4) at 0.25 h. This molecule was more active and showed longer duration of satisfactory action than Phenytoin. The other compounds **5j** and **5o** were moderately effective in rat MES oral screen and protected only 50% (2/4) of tested animals at the time point 0.5 h (**5j**) or 4 h (**5o**) and 25% (1/4) at 1 h (**5j**). All derivatives tested were non-neurotoxic when given orally. The *in vivo* data in rats confirmed absorption of compounds from gastrointestinal tract and also their penetration to central nervous system. The inhibition of electrically induced seizures that is characteristic for Phenytoin and Phenytoin-like drugs may indicate the influence of compounds on voltage depended Na + channels as the most plausible mechanism of antiepileptic action.

Table 3
Antiepileptic activity and toxicity of compounds **5c**, **5g**, **5j**, **5n** and **5o** administered orally (30 mg/kg) to rats.

Compds	MES[a]					TOX[b]
	0.25 h[c]	0.5 h[c]	1 h[c]	2 h[c]	4 h[c]	
5c	1/4	2/4	2/4	4/4	4/4	0/4 (−)[d]
5g	1/4	2/4	2/4	4/4	4/4	0/4 (−)[d]
5j	0/4	2/4	1/4	0/4	0/4	0/4 (−)[d]
5n	1/4	2/4	2/4	4/4	4/4	0/4 (−)[d]
5o	0/4	0/4	0/4	0/4	2/4	0/4 (−)[d]
Phenytoin[e]	1/4	4/4	3/4	3/4	3/4	0/4 (−)[d]

[a] Maximal electroshock test (dose of 30 mg/kg was administrated. The data indicate: number of rats protected/number of rats tested).
[b] Neurotoxicity (number of rats protected/number of rats tested).
[c] Time after drug administration.
[d] (−) No neurotoxicity at dose tested.
[e] Reference drug, data for phenytoin ref [23].

4.5. Structure activity Relationships (SAR) analysis

On correlating the structures of the sample candidate with their biological activity, it has been observed that, for the 18 phenyl substituted derivatives **5c**–**5t**, four compounds (**5c**, **5g**, **5j** and **5n**) has selectivity towards MES (at 30 mg/kg) and one compound (**5o**) had *sc*PTZ activity (at 30–100 mg/kg). All the above mentioned five compounds were all *p*-substituted. The position of the substituted group on the phenyl ring appeared to greatly influence the antiepileptic activity; the *p*-chloro derivative **5c** exhibited higher antiepileptic activity than the *o*-chloro derivative **5e**. At the same *p*-position, the compound with fluorine substitution **5g** and **5h** exhibited higher antiepileptic activity than the compound with nitro substitution **5i.** Moreover, *p*–OCH_3 and *p*–CH_3 substituted compounds **5q** and **5s** had slighter lower activity. However, the unsubstituted phenyl **5a**, **5b** and the phenyl ring substituted by *m*-nitro **5k**, **5l** compounds did not exhibit significant antiepileptic activity. In this series, generally compounds with morpholine ring exhibited significant antiepileptic activity in comparison to piperidine ring. The increases in antiepileptic activity of test compounds with morpholine derivatives may be attributed due to the presence of extra one electronegative oxygen atom on morpholine (which is absent in piperidine) ring which might be accountable for additional hydrogen bonding with the binding site.

5. Conclusion

We have designed and synthesized the title compounds while remembering the fact that a majority of clinically active antiepileptics possess a nitrogen hetero atomic system with one or two phenyl rings, at least one carbonyl group in their structure and presence of hydrogen donor/Acceptor unit. The structure of the title compounds **5a**–**5t** satisfied all the pharmacophoric structural requirements that is, presence of indole-2,3-dione moiety as hydrophobic portion, N and O as electron donor system, presence of carbonyl group and another hydrophobic distal aryl ring responsible for controlling the pharmacokinetic properties of the antiepileptics. All the twenty compounds are screened for their antiepileptic activity by MES and *sc*PTZ model along with its neurotoxicity. Among the screened compounds, **5c**, **5g**, **5j** and **5n** were found significant in MES screening, while compound **5o** showed significant antiepileptic activity in *sc*PTZ model. These five compounds were selected for oral administration in rats at 30 mg/kg dose. Compounds **5c**, **5g** and **5n** exhibited better antiepileptic activity in oral dose than standard drug phenytoin. The most active was 1-(4-chlorobenzylidene)-3-(1-(morpholinomethyl)-2,3-dioxoindolin-5-yl)urea (**5c**) that revealed protection in the electrically induced seizures at a dose of 30 mg/kg and 100 mg/kg 0.5 h and 4 h after *i.p.* administration respectively. This molecule provided also protection in the *sc*PTZ at a dose of 300 mg/kg in both time intervals. Compound **5c** emerged out as the lead molecule with a wide spectrum of antiepileptic activity without any neurotoxicity.

6. Experimental protocols

6.1. Chemistry

6.1.1. General remarks

The chemicals and reagents used were obtained from various chemical units Qualigens, E. Merck India Ltd., CDH, and SD Fine Chem. These solvents used were of LR grade and purified before their use. The silica gel G used for analytical chromatography (TLC) was obtained from E. Merck India Ltd. Solvent systems were used Toluene: Ethyl acetate: Formic acid (5:4:1). All the melting points

were taken in open glass capillary and are uncorrected. ^{1}H NMR spectra were taken on a Bruker ultra shield (400 MHz) NMR spectrometer in $CDCl_3$ using tetramethylsilane [$(CH_3)_4Si$] as internal standard. Chemical shift (δ) are expressed in ppm. Mass spectra were obtained on a JEOL-SX-102 instrument using electron impact ionization. All the IR spectra were recorded in KBr pellets on a Jasco FT-IR 410 spectrometer. Elemental analyses were performed on a Perkine Elmer model 240c analyzer and were within ±0.4% of the theoretical values.

*6.1.2. General procedure for the synthesis of title compounds (**5a**–**5t**)*

*6.1.2.1. Preparation of 5-nitroisatin (**1**).* The 5-nitroisatin (**1**) was prepared according to the reported literature [25]. Briefly, in a mixture of 50 g (35 mL, 0.50 mol) of conc. nitric acid and 74 g (40 mL, 0.75 mol) conc. sulfuric acid, isatin (48.50 g, 0.33 mol) was added slowly with frequent shaking in 500 mL round bottomed flask. Then the mixture was cooled by immersing the flask in crushed ice cold water. After adding all isatin, flask was fitted with reflux condenser and the mixture was heated on water bath maintaining the temperature at 60 °C for 1 h to obtain the desired compound 5-nitroisatin (**1**). Then the entire content was then transferred into a beaker containing 500 mL cold water, stirred in order to wash out as much acid from the desired product. When compound **1** settled completely to the bottom, the upper acid layer was removed from the mixture. Then the bottom layer was transferred to the separating funnel and shaked vigorously with about 50 mL of water. Then the residual layer was collected, dried with anhydrous calcium chloride and finally filtered to obtain the pure compound (1). Yield 65%, Mp 230 °C; IR (KBr) cm^{-1}: 3342 (NH_{str}), 2996 (Ar–CH_{str}), 1732 (C=O, Isatin), 1570 (Ar–NO_2, N=O asym); 1348 (Ar–NO_2, N=O sym); ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 7.02–7.94 (m, 3H, Ar–H), 8.92 (s, 1H, NH-Isatin); MS (EI) *m*/*z* 192 [M$^+$]; Anal. Calcd for $C_8H_4N_2O_4$: C, 50.01; H, 2.10; N, 14.58. Found: C, 49.91; H, 2.12; N, 14.62.

*6.1.2.2. 5-Nitro-1-substituted indolin-2,3-dione (**2a**–**2b**).* To the solution of 5-nitro indolin-2-one (**1**) (0.04 mol) in 95% absolute ethanol (100 mL), aqueous formaldehyde 37% (1.0 mL) was added. Then secondary amino compound (0.04 mol) (morpholine/piperidine) was added drop-wise slowly to the above solution under stirring. After the addition was over, the entire reaction mixture was stirred at room temperature for 3 h, and then kept aside for 48 h in refrigerator to form crystals. Finally the products in the form of crystals were separated by filtration, washed with hexane, and vacuum dried. Desired compounds were finally recrystallized with ethanol to obtain pure product.

*6.1.2.2.1. 1-(morpholinomethyl)-5-nitro indoline-2,3-dione (**2a**).* Yield 68%, Mp 248 °C; IR (KBr) cm^{-1}: 3018 (Ar–CH_{str}), 1734 (C=O, Isatin), 1565 (Ar–NO_2, N=O asym); 1352 (Ar–NO_2, N=O sym), 1070 (Cyclic C–O–C_{str}); ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 2.45 (t, J = 6.0 Hz, 4H, –CH_2 morpholine); 3.32 (t, J = 5.6 Hz, 4H, –CH_2 morpholine); 4.28 (s, 2H, –CH_2); 7.40–7.86 (m, 3H, Ar–H); MS (EI) *m*/*z* 291 [M$^+$]; Anal. Calcd for $C_{13}H_{13}N_3O_5$: C, 53.61; H, 4.50; N, 14.43. Found: C, 53.78; H, 4.54; N, 14.38.

*6.1.2.2.2. 5-Nitro-1-(piperidin-1-ylmethyl)indoline-2,3-dione (**2b**).* Yield 56%, Mp 221 °C; IR (KBr) cm^{-1}: 3024 (Ar–CH_{str}), 1710 (C=O, Isatin), 1544 (Ar–NO_2, N=O asym); 1332 (Ar–NO_2, N=O asym); ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 1.45–1.56 (m, 6H, –CH_2 piperidinyl); 2.35–2.44 (m, 4H, –CH_2 piperidinyl); 4.40 (s, 2H, –CH_2); 7.20–7.64 (m, 3H, Ar–H); MS (EI) *m*/*z* 289 [M$^+$]; Anal. Calcd for $C_{14}H_{15}N_3O_4$: C, 58.13; H, 5.23; N, 14.53. Found: C, 58.30; H, 5.25; N, 14.58.

*6.1.2.3. 5-Amino-1-substituted indolin-2,3-dione (**3a**–**3b**).* In a round bottomed flask containing 200 mL of absolute ethanol, compound (**2a** or **2b**) (0.01 mol) and iron powder (0.01 mol) was added. After the addition was over, the mixture was heated on a oil bath until the temperature reaches to 80–85 °C. After that, in the reaction flask 4 mL of hydrochloric acid (1.2 M) was added and the content was stirred in the same temperature for 4 h. Finally the slurry was filtered and pH of the filtrate was adjusted to 7–8 with the help of sodium bicarbonate to get the precipitate of compound **3a** or **3b**. Recrystallization from ethanol rendered desired products in pure form.

*6.1.2.3.1. 1-(morpholinomethyl)-5-amino indoline-2,3-dione (**3a**).* Yield 61%, Mp 226 °C; IR (KBr) cm^{-1}: 3386 (NH_{str}), 3128 (Ar–CH_{str}), 1740 (C=O, Isatin), 1055 (Cyclic C–O–C_{str}); ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 2.38 (t, J = 6.0 Hz, 4H, –CH_2 morpholine); 3.38 (t, J = 5.6 Hz, 4H, –CH_2 morpholine); 3.86 (s, 2H, –NH_2), 4.24 (s, 2H, –CH_2); 7.42–7.76 (m, 3H, Ar–H); MS (EI) *m*/*z* 261 [M$^+$]; Anal. Calcd for $C_{13}H_{15}N_3O_3$: C, 59.76; H, 5.79; N, 16.08. Found: C, 59.94; H, 5.81; N, 16.13.

*6.1.2.3.2. 5-Amino-1-(piperidin-1-ylmethyl)indoline-2,3-dione (**3b**).* Yield 54%, Mp 234 °C; IR (KBr) cm^{-1}: 3398 (NH_{str}), 3089 (Ar–CH_{str}), 1722 (C=O, Isatin); ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 1.34–1.47 (m, 6H, –CH_2 piperidinyl); 2.29–2.38 (m, 4H, –CH_2 piperidinyl); 3.74 (s, 2H, –NH_2), 4.35 (s, 2H, –CH_2); 7.36–7.68 (m, 3H, Ar–H); MS (EI) *m*/*z* 259 [M$^+$]; Anal. Calcd for $C_{14}H_{17}N_3O_2$: C, 64.85; H, 6.61; N, 16.20. Found: C, 65.05; H, 6.63; N, 16.26.

*6.1.2.4. 1-(1-Substituted 2,3-dioxoindolin-5-yl) urea (**4a**–**4b**).* Compound **3a**, **3b** (0.01 mol) was dissolved in 10 mL glacial acetic acid and the volume was diluted to 100 mL with the same in a conical flask. To it a solution of NaOCN (Sodium cyanate) (0.01 mol) and 50 mL of warm water was then added slowly with continuous stirring. Then the reaction mixture was allowed to stand for 30 min, and then cooled in crushed ice, allowed to stand for further 30 min. The product thus obtained was filtered and washed with cold water, finally dried at 100 °C. The product was recrystallized at least once from ethanol to give the pure form.

*6.1.2.4.1. 1-(1-(morpholinomethyl)-2,3-dioxoindolin-5-yl) urea (**4a**).* Yield 72%, Mp 272 °C; IR (KBr) cm^{-1}: 3364 (NH_{str}), 3096 (Ar–CH_{str}), 1746 (C=O, Isatin), 1648 (C=O, Urea), 1030 (Cyclic C–O–C_{str}); ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 2.36 (t, J = 6.0 Hz, 4H, –CH_2 morpholine); 3.34 (t, J = 5.6 Hz, 4H, –CH_2 morpholine); 3.76 (s, 2H, –NH_2), 4.36 (s, 2H, –CH_2); 7.32–7.72 (m, 3H, Ar–H); 8.42 (s, 1H, NH-Urea); MS (EI) *m*/*z* 304 [M$^+$]; Anal. Calcd for $C_{14}H_{16}N_4O_4$: C, 55.26; H, 5.30; N, 18.41. Found: C, 55.44; H, 5.32; N, 18.48.

*6.1.2.4.2. 1-(2,3-Dioxo-1-(piperidin-1-ylmethyl)indolin-5-yl)urea (**4b**).* Yield 71%, Mp 246 °C; IR (KBr) cm^{-1}: 3398 (NH_{str}), 3120 (Ar–CH_{str}), 1734 (C=O, Isatin), 1668 (C=O, Urea); ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 1.28–1.35 (m, 6H, –CH_2 piperidinyl); 2.48–2.56 (m, 4H, –CH_2 piperidinyl); 3.88 (s, 2H, –NH_2), 4.24 (s, 2H, –CH_2); 7.20–7.68 (m, 3H, Ar–H); 8.50 (s, 1H, NH-Urea); MS (EI) *m*/*z* 302 [M$^+$]; Anal. Calcd for $C_{15}H_{18}N_4O_3$: C, 59.59; H, 6.00; N, 18.53. Found: C, 60.10; H, 6.02; N, 18.60.

*6.1.2.5. 1-(substituted benzylidene)-3-(1-(morpholino/piperidino methyl)-2,3-dioxoindolin-5-yl) urea (**5a**–**5t**).* Title compounds (**5a**–**5t**) was synthesized by adding 1-(1- morpholino/piperidino methyl 2,3-dioxoindolin-5-yl) urea **4a**, **4b** (0.01 mol) in fraction with the well stirred mixture of different aromatic aldehydes (0.01 mol) in ethanol 50 mL and few mL of glacial acetic acid. Then this mixture was refluxed for 8 h and kept aside. The product that separated out was filtered, dried and recrystallized from ethanol. The method used for the preparation and isolation of the compounds gave materials of good purity, as evidenced by their spectral analyses and by thin layer chromatography. The title compounds are found to be soluble in chloroform, dimethyl sulfoxide, and dimethylformamide.

*6.1.2.5.1. 1-Benzylidene-3-(1-(morpholinomethyl)-2,3-dioxoindolin-5-yl)urea (**5a**).* FT-IR (KBr): cm^{-1} 3352 (NH_{Str}); 3038 (Ar C–H_{Str}); 1710 (C=O, isatin); 1564 (CH=N); 1059 (Cyclic C–O–C_{str}); ^1H NMR (400 MHz, $CDCl_3$, δ ppm): 2.56 (t, J = 6.2 Hz, 4H, $-CH_2$ morpholine); 3.30 (t, J = 5.8 Hz, 4H, $-CH_2$ morpholine); 4.12 (s, 2H, $-CH_2$); 6.80–7.52 (m, 8H, Ar–CH); 8.12 (s, 1H, NH); 8.52 (s, 1H, CH=N); MS (EI) m/z: 392 [M^+]; Anal. Calcd for $C_{21}H_{20}N_4O_4$: C, 64.28; H, 5.14; N, 14.28. Found: C, 64.12; H, 5.16; N, 14.33.

*6.1.2.5.2. 1-Benzylidene-3-(2,3-dioxo-1-(piperidin-1-ylmethyl) indolin-5-yl)urea (**5b**).* FT-IR (KBr): cm^{-1} 3386 (NH_{Str}); 3012 (Ar C–H_{Str}); 1742 (C=O, isatin); 1574 (CH=N); ^1H NMR (400 MHz, $CDCl_3$, δ ppm): 1.20–1.52 (m, 6H, $-CH_2$ piperidinyl); 2.21–2.48 (m, 4H, $-CH_2$ piperidinyl); 4.44 (s, 2H, $-CH_2$); 7.10–7.84 (m, 8H, Ar–H); 8.42 (s, 1H, NH-Urea); 8.58 (s, 1H, CH=N); MS (EI) m/z 390 [M^+]; Anal. Calcd for $C_{22}H_{22}N_4O_3$: C, 67.68; H, 5.68; N, 14.35. Found: C, 67.81; H, 5.70; N, 14.40.

*6.1.2.5.3. 1-(4-chlorobenzylidene)-3-(1-(morpholinomethyl)-2,3-dioxoindolin-5-yl)urea (**5c**).* FT-IR (KBr): cm^{-1} 3366 (NH_{Str}); 3058 (Ar C–H_{Str}); 1726 (C=O, isatin); 1588 (CH=N,); 1068 (Cyclic C–O–C_{str}); 850 (C–Cl); ^1H NMR (400 MHz, $CDCl_3$, δ ppm): 2.55 (t, J = 6.2 Hz, 4H, $-CH_2$ morpholine); 3.28 (t, J = 5.8 Hz, 4H, $-CH_2$ morpholine); 4.30 (s, 2H, $-CH_2$); 6.88–8.10 (m, 7H, Ar–CH); 8.22 (s, 1H, NH); 8.78 (s, 1H, CH=N); MS (EI) m/z: 428 [M+2]; Anal. Calcd for $C_{21}H_{19}ClN_4O_4$: C, 59.09; H, 4.49; N, 13.13. Found: C, 58.87; H, 4.51; N, 13.18.

*6.1.2.5.4. 1-(4-chlorobenzylidene)-3-(2,3-dioxo-1-(piperidin-1-ylmethyl) indolin-5-yl)urea (**5d**).* FT-IR (KBr): cm^{-1} 3358 (NH_{Str}); 3016 (Ar C–H_{Str}); 1722 (C=O, isatin); 1562 (CH=N); 876 (C–Cl); ^1H NMR (400 MHz, $CDCl_3$, δ ppm): 1.23–1.51 (m, 6H, $-CH_2$ piperidinyl); 2.34–2.61 (m, 4H, $-CH_2$ piperidinyl); 4.10 (s, 2H, $-CH_2$); 7.23–7.94 (m, 7H, Ar–H); 8.22 (s, 1H, NH-Urea); 8.48 (s, 1H, CH=N); MS (EI) m/z: 426 [M+2]; Anal. Calcd for $C_{22}H_{21}ClN_4O_3$: C, 62.19; H, 4.98; N, 13.19. Found: C, 62.36; H, 5.00; N, 13.14.

*6.1.2.5.5. 1-(2-chlorobenzylidene)-3-(1-(morpholinomethyl)-2,3-dioxoindolin-5-yl)urea (**5e**).* FT-IR (KBr): cm^{-1} 3376 (NH_{Str}); 3024 (Ar C–H_{Str}); 1732 (C=O, isatin); 1590 (CH=N); 1064 (Cyclic C–O–C_{str}); 765 (C–Cl); ^1H NMR (400 MHz, $CDCl_3$, δ ppm): 2.54 (t, J = 6.0 Hz, 4H, $-CH_2$ morpholine); 3.26 (t, J = 5.8 Hz, 4H, $-CH_2$ morpholine); 4.48 (s, 2H, $-CH_2$); 7.23–8.00 (m, 7H, Ar–CH); 8.18 (s, 1H, NH); 8.42 (s, 1H, CH=N); MS (EI) m/z: 428 [M+2]; Anal. Calcd for $C_{21}H_{19}ClN_4O_4$: C, 59.09; H, 4.49; N, 13.13. Found: C, 59.23; H, 4.51; N, 13.17.

*6.1.2.5.6. 1-(2-chlorobenzylidene)-3-(2,3-dioxo-1-(piperidin-1-ylmethyl) indolin-5-yl)urea (**5f**).* FT-IR (KBr): cm^{-1} 3350 (NH_{Str}); 2986 (Ar C–H_{Str}); 1718 (C=O, isatin); 1546 (CH=N); 748 (C–Cl); ^1H NMR (400 MHz, $CDCl_3$, δ ppm): 1.19–1.47 (m, 6H, $-CH_2$ piperidinyl); 2.44–2.72 (m, 4H, $-CH_2$ piperidinyl); 4.16 (s, 2H, $-CH_2$); 7.04–7.84 (m, 7H, Ar–H); 8.11 (s, 1H, NH-Urea); 8.54 (s, 1H, CH=N); MS (EI) m/z: 426 [M+2]; Anal. Calcd for $C_{22}H_{21}ClN_4O_3$: C, 62.19; H, 4.98; N, 13.19. Found: C, 62.39; H, 4.96; N, 13.14.

*6.1.2.5.7. 1-(4-fluorobenzylidene)-3-(1-(morpholinomethyl)-2,3-dioxoindolin-5-yl)urea (**5g**).* FT-IR (KBr): cm^{-1} 3392 (NH_{Str}); 3022 (Ar C–H_{Str}); 1744 (C=O, isatin); 1570 (CH=N); 1067 (Cyclic C–O–C_{str}); 1112 (C–F); ^1H NMR (400 MHz, $CDCl_3$, δ ppm): 2.40 (t, J = 5.6 Hz, 4H, $-CH_2$ morpholine); 3.36 (t, J = 5.6 Hz, 4H, $-CH_2$ morpholine); 4.22 (s, 2H, $-CH_2$); 6.98–7.84 (m, 7H, Ar–CH); 8.16 (s, 1H, NH); 8.64 (s, 1H, CH=N); MS (EI) m/z: 410 [M^+]; Anal. Calcd for $C_{21}H_{19}FN_4O_4$: C, 61.46; H, 4.67; N, 13.65. Found: C, 61.67; H, 4.69; N, 13.60.

*6.1.2.5.8. 1-(4-fluorobenzylidene)-3-(2,3-dioxo-1-(piperidin-1-ylmethyl) indolin-5-yl)urea (**5h**).* FT-IR (KBr): cm^{-1} 3326 (NH_{Str}); 3032 (Ar C–H_{Str}); 1736 (C=O, isatin); 1566 (CH=N); 1126 (C–F); ^1H NMR (400 MHz, $CDCl_3$, δ ppm): 1.45–1.76 (m, 6H, $-CH_2$ piperidinyl); 2.49–2.81 (m, 4H, $-CH_2$ piperidinyl); 3.98 (s, 2H, $-CH_2$); 7.12–7.77 (m, 7H, Ar–H); 8.33 (s, 1H, NH-Urea); 8.51 (s, 1H, CH=N); MS (EI) m/z: 408 [M^+]; Anal. Calcd for $C_{22}H_{21}FN_4O_3$: C, 64.70; H, 5.18; N, 13.72. Found: C, 64.83; H, 5.20; N, 13.77.

*6.1.2.5.9. 1-(4-nitrobenzylidene)-3-(1-(morpholinomethyl)-2,3-dioxoindolin-5-yl)urea (**5i**).* FT-IR (KBr): cm^{-1} 3321 (NH_{Str}); 3045 (Ar C–H_{Str}); 1720 (C=O, isatin); 1584 (CH=N); 1526 (Ar–NO_2, N=O asym); 1344 (Ar–NO_2, N=O sym); 1041 (Cyclic C–O–C_{str}); ^1H NMR (400 MHz, $CDCl_3$, δ ppm): 2.44 (t, J = 6.2 Hz, 4H, $-CH_2$ morpholine); 3.32 (t, J = 5.8 Hz, 4H, $-CH_2$ morpholine); 4.10 (s, 2H, $-CH_2$); 6.84–7.96 (m, 7H, Ar–CH); 8.20 (s, 1H, NH); 8.54 (s, 1H, CH=N); MS (EI) m/z: 437 [M^+]; Anal. Calcd for $C_{21}H_{19}N_5O_6$: C, 57.66; H, 4.38; N, 16.01. Found: C, 57.78; H, 4.40; N, 16.07.1528 (Ar–NO2, N]O assym.); 1348 (Ar–NO2, N]O sym.);

*6.1.2.5.10. 1-(4- nitrobenzylidene)-3-(2,3-dioxo-1-(piperidin-1-ylmethyl) indolin-5-yl)urea (**5j**).* FT-IR (KBr): cm^{-1} 3372 (NH_{Str}); 3051 (Ar C–H_{Str}); 1744 (C=O, isatin); 1596 (CH=N); 1548 (Ar–NO_2, N=O asym); 1356 (Ar–NO_2, N=O sym); 860 (C–Cl); ^1H NMR (400 MHz, $CDCl_3$, δ ppm): 1.40–1.72 (m, 6H, $-CH_2$ piperidinyl); 2.30–2.64 (m, 4H, $-CH_2$ piperidinyl); 4.40 (s, 2H, $-CH_2$); 6.60–7.74 (m, 7H, Ar–H); 8.25 (s, 1H, NH-Urea); 8.65 (s, 1H, CH=N); MS (EI) m/z: 435 [M^+]; Anal. Calcd for $C_{22}H_{21}N_5O_5$: C, 60.68; H, 4.86; N, 16.08. Found: C, 60.90; H, 4.88; N, 16.03.

*6.1.2.5.11. 1-(3-nitrobenzylidene)-3-(1-(morpholinomethyl)-2,3-dioxoindolin-5-yl)urea (**5k**).* FT-IR (KBr): cm^{-1} 3378 (NH_{Str}); 3050 (Ar C–H_{Str}); 1732 (C=O, isatin); 1556 (CH=N); 1568 (Ar–NO_2, N=O asym); 1326 (Ar–NO_2, N=O sym); 1063 (Cyclic C–O–C_{str}); ^1H NMR (400 MHz, $CDCl_3$, δ ppm): 2.52 (t, J = 6.2 Hz, 4H, $-CH_2$ morpholine); 3.30 (t, J = 5.8 Hz, 4H, $-CH_2$ morpholine); 4.20 (s, 2H, $-CH_2$); 7.02–7.98 (m, 7H, Ar–CH); 8.14 (s, 1H, NH); 8.38 (s, 1H, CH=N); MS (EI) m/z: 437 [M^+]; Anal. Calcd for $C_{21}H_{19}N_5O_6$: C, 57.66; H, 4.38; N, 16.01. Found: C, 57.48; H, 4.36; N, 16.06.

*6.1.2.5.12. 1-(3- nitrobenzylidene)-3-(2,3-dioxo-1-(piperidin-1-ylmethyl) indolin-5-yl)urea (**5l**).* FT-IR (KBr): cm^{-1} 3384 (NH_{Str}); 3062 (Ar C–H_{Str}); 1730 (C=O, isatin); 1567 (CH=N); 1560 (Ar–NO_2, N=O asym); 1332 (Ar–NO_2, N=O sym); ^1H NMR (400 MHz, $CDCl_3$, δ ppm): 1.33–1.74 (m, 6H, $-CH_2$ piperidinyl); 2.50–2.82 (m, 4H, $-CH_2$ piperidinyl); 4.31 (s, 2H, $-CH_2$); 6.90–7.78 (m, 7H, Ar–H); 8.19 (s, 1H, NH-Urea); 8.45 (s, 1H, CH=N); MS (EI) m/z: 435 [M^+]; Anal. Calcd for $C_{22}H_{21}N_5O_5$: C, 60.68; H, 4.86; N, 16.08. Found: C, 60.48; H, 4.84; N, 16.02.

*6.1.2.5.13. 1-(4-bromobenzylidene)-3-(1-(morpholinomethyl)-2,3-dioxoindolin-5-yl)urea (**5m**).* FT-IR (KBr): cm^{-1} 3358 (NH_{Str}); 3010 (Ar C–H_{Str}); 1712 (C=O, isatin); 1578 (CH=N); 1032 (Cyclic C–O–C_{str}); 632 (C–Br); ^1H NMR (400 MHz, $CDCl_3$, δ ppm): 2.55 (t, J = 5.8 Hz, 4H, $-CH_2$ morpholine); 3.36 (t, J = 5.6 Hz, 4H, $-CH_2$ morpholine); 4.45 (s, 2H, $-CH_2$); 7.13–7.85 (m, 7H, Ar–CH); 8.13 (s, 1H, NH); 8.60 (s, 1H, CH=N); MS (EI) m/z: 472 [M+2]; Anal. Calcd for $C_{21}H_{19}BrN_4O_4$: C, 53.52; H, 4.06; N, 11.89. Found: C, 53.36; H, 4.04; N, 11.93.

*6.1.2.5.14. 1-(4-bromobenzylidene)-3-(2,3-dioxo-1-(piperidin-1-ylmethyl) indolin-5-yl)urea (**5n**).* FT-IR (KBr): cm^{-1} 3365 (NH_{Str}); 3021 (Ar C–H_{Str}); 1741 (C=O, isatin); 1539 (CH=N); 662 (C–Br); ^1H NMR (400 MHz, $CDCl_3$, δ ppm): 1.11–1.50 (m, 6H, $-CH_2$ piperidinyl); 2.15–2.60 (m, 4H, $-CH_2$ piperidinyl); 4.12 (s, 2H, $-CH_2$); 6.90–7.82 (m, 7H, Ar–H); 8.30 (s, 1H, NH-Urea); 8.64 (s, 1H, CH=N); MS (EI) m/z: 470 [M+2]; Anal. Calcd for $C_{22}H_{21}BrN_4O_3$: C, 56.30; H, 4.51; N, 11.94. Found: C, 56.48; H, 4.53; N, 11.98.

*6.1.2.5.15. 1-(4-hydroxybenzylidene)-3-(1-(morpholinomethyl)-2,3-dioxoindolin-5-yl)urea (**5o**).* FT-IR (KBr): cm^{-1} 3510 (OH_{str}); 3362 (NH_{Str}); 3020 (Ar C–H_{Str}); 1743 (C=O, isatin); 1546 (CH=N); 1056 (Cyclic C–O–C_{str}); ^1H NMR (400 MHz, $CDCl_3$, δ ppm): 2.50 (t, J = 6.0 Hz, 4H, $-CH_2$ morpholine); 3.32 (t, J = 5.6 Hz, 4H, $-CH_2$ morpholine); 4.55 (s, 2H, $-CH_2$); 5.02 (s, 1H, OH); 6.92–7.82 (m, 7H, Ar–CH); 8.35 (s, 1H, NH); 8.74 (s, 1H, CH=N); MS (EI) m/z: 408 [M^+]; Anal. Calcd for $C_{21}H_{20}N_4O_5$: C, 61.76; H, 4.94; N, 13.72. Found: C, 61.70; H, 4.96; N, 13.77.

*6.1.2.5.16. 1-(4-hydroxybenzylidene)-3-(2,3-dioxo-1-(piperidin-1-ylmethyl) indolin-5-yl)urea (**5p**).* FT-IR (KBr): cm^{-1} 3526 (OH_{str}); 3365 (NH_{Str}); 3008 (Ar C–H_{Str}); 1756 (C=O, isatin); 1562 (CH=N); ^1H NMR (400 MHz, $CDCl_3$, δ ppm): 1.37–1.69 (m, 6H, $-CH_2$ piperidinyl); 2.48–2.89 (m, 4H, $-CH_2$ piperidinyl); 4.45 (s, 2H, $-CH_2$); 5.20 (s, 1H, OH); 7.06–7.86 (m, 7H, Ar–H); 8.22 (s, 1H, NH-Urea); 8.51 (s, 1H, CH=N); MS (EI) *m/z*: 406 [M^+]; Anal. Calcd for $C_{22}H_{22}N_4O_4$: C, 65.01; H, 5.46; N, 13.78. Found: C, 65.20; H, 5.48; N, 13.84.

*6.1.2.5.17. 1-(4-methoxybenzylidene)-3-(1-(morpholinomethyl)-2,3-dioxoindolin-5-yl)urea (**5q**).* FT-IR (KBr): cm^{-1} 3369 (NH_{Str}); 3014 (Ar C–H_{Str}); 1722 (C=O, isatin); 1550 (CH=N); 1084 (Cyclic C–O–C_{str}); ^1H NMR (400 MHz, $CDCl_3$, δ ppm): 2.45 (s, 3H, OCH_3); 2.56 (t, $J = 6.2$ Hz, 4H, $-CH_2$ morpholine); 3.36 (t, $J = 5.6$ Hz, 4H, $-CH_2$ morpholine); 4.04 (s, 2H, $-CH_2$); 6.90–7.88 (m, 7H, Ar–CH); 8.24 (s, 1H, NH); 8.42 (s, 1H, CH=N); MS (EI) *m/z*: 422 [M^+]; Anal. Calcd for $C_{22}H_{22}N_4O_5$: C, 62.55; H, 5.25; N, 13.26. Found: C, 62.72; H, 5.27; N, 13.31.

*6.1.2.5.18. 1-(4-methoxybenzylidene)-3-(2,3-dioxo-1-(piperidin-1-ylmethyl) indolin-5-yl)urea (**5r**).* FT-IR (KBr): cm^{-1} 3390 (NH_{Str}); 3012 (Ar C–H_{Str}); 1715 (C=O, isatin); 1586 (CH=N); ^1H NMR (400 MHz, $CDCl_3$, δ ppm): 1.32–1.65 (m, 6H, $-CH_2$ piperidinyl); 2.16–2.42 (m, 4H, $-CH_2$ piperidinyl); 2.70 (s, 3H, OCH_3); 4.31 (s, 2H, $-CH_2$); 7.14–7.92 (m, 7H, Ar–H); 8.30 (s, 1H, NH-Urea); 8.45 (s, 1H, CH=N); MS (EI) *m/z*: 420 [M^+]; Anal. Calcd for $C_{23}H_{24}N_4O_4$: C, 65.70; H, 5.75; N, 13.33. Found: C, 65.93; H, 5.77; N, 13.37.

*6.1.2.5.19. 1-(4-methylbenzylidene)-3-(1-(morpholinomethyl)-2,3-dioxoindolin-5-yl)urea (**5s**).* FT-IR (KBr): cm^{-1} 3356 (NH_{Str}); 3016 (Ar C–H_{Str}); 1726 (C=O, isatin); 1572 (CH=N); 1045 (Cyclic C–O–C_{str}); ^1H NMR (400 MHz, $CDCl_3$, δppm): 2.28 (s, 3H, CH_3); 2.54 (t, $J = 6.0$ Hz, 4H, $-CH_2$ morpholine); 3.34 (t, $J = 5.6$ Hz, 4H, $-CH_2$ morpholine); 4.08 (s, 2H, $-CH_2$); 6.92–7.90 (m, 7H, Ar–CH); 8.16 (s, 1H, NH); 8.44 (s, 1H, CH=N); MS (EI) *m/z*: 406 [M^+]; Anal. Calcd for $C_{22}H_{22}N_4O_4$: C, 65.01; H, 5.46; N, 13.78. Found: C, 65.21; H, 5.48; N, 13.82.

*6.1.2.5.20. 1-(4-methylbenzylidene)-3-(2,3-dioxo-1-(piperidin-1-ylmethyl) indolin-5-yl)urea (**5t**).* FT-IR (KBr): cm^{-1} 3348 (NH_{Str}); 3046 (Ar C–H_{Str}); 1728 (C=O, isatin); 1548 (CH=N); ^1H NMR (400 MHz, $CDCl_3$, δ ppm): 1.41–1.73 (m, 6H, $-CH_2$ piperidinyl); 2.35 (s, 3H, CH_3); 2.56–2.89 (m, 4H, $-CH_2$ piperidinyl); 4.39 (s, 2H, $-CH_2$); 7.00–7.85 (m, 7H, Ar–H); 8.25 (s, 1H, NH-Urea); 8.50 (s, 1H, CH=N); MS (EI) *m/z*: 404 [M^+]; Anal. Calcd for $C_{23}H_{24}N_4O_3$: C, 68.30; H, 5.98; N, 13.85. Found: C, 68.53; H, 6.00; N, 13.90.

6.2. Pharmacology

6.2.1. The maximal electroshock test (MES)

The MES is a model for generalized tonic-clonic seizures and provides a hint of a compound's ability to stop seizure spread when all neuronal circuits in the brain are maximally active. These seizures are extremely reproducible and are electro physiologically reliable with human seizures. For the MES, a drop of anesthetic and electrolyte solution (tetracaine hydrochloride (0.5%) in saline (0.9%)) was applied to the eyes of individual animal before to placement of the corneal electrodes. The electrical stimulus in the MES test was 50 mA, 60 Hz, for mice and 150 mA, 60 Hz, for rats delivered for 0.2 s by an apparatus similar to that initially described by Woodbury and Davenport [26,27]. Abolition of the hindleg tonic extensor component of the seizure was used as the endpoint. Mice are initially tested with different doses of 30, 100 and 300 mg/kg of test compound given by *i.p.* injection at various intervals while rats are initially screened at a fixed dose of 30 mg/kg given by oral route.

6.2.2. The subcutaneous pentylenetetrazole seizure test (scPTZ)

Subcutaneous injection of the convulsant Pentylenetetrazole produces clonic seizures in laboratory animals. The scPTZ test detects the ability of test compounds to raise the seizure threshold of an animal and thus protect it from exhibiting a clonic seizure. Animals are pretreated with various doses of the test compound given by *i.p.* injection. The dose of Pentylenetetrazole which induce convulsions in 97% of animals (CD_{97}: 85 mg/kg mice) is injected into a loose fold of skin in the midline of the neck. The animals are placed in isolation cages to minimize stress [28] and observed for the next 30 min for the presence or absence of a seizure. An episode of clonic spasms, approximately 3–5 s, of the fore and/or hindlimbs, jaws, or vibrissae is taken as the endpoint. Animals which do not meet this criterion are considered protected.

6.2.3. Acute toxicity-minimal motor impairment

To assess a compound's undesirable side effects (toxicity), animals are monitored for overt signs of impaired neurological or muscular function. In mice, the rotorod [29] procedure is used to disclose minimal muscular (MMI) or neurological impairment (MNI). When a mouse is placed on a rod that rotates at a speed of 6 rpm, the animal can maintain its equilibrium for long periods of time. The animal is considered toxic if it falls off this rotating rod three times during a 1 min period. In addition to MMI, animals may exhibit a circular or zigzag gait, abnormal body posture and spread of the legs, tremors, hyperactivity, lack of exploratory behavior, somnolence, stupor, catalepsy, loss of placing response and changes in muscle tone.

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Appendix. Supplementary material

Supplementary data related to this article can be found online at doi:10.1016/j.ejmech.2011.10.020.

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